



# Prevalence and Molecular Detection of *Escherichia Coli* Isolated from Raw Cow Milk at Selected Collection Centers in Bushenyi District, Western Uganda: A Potential Risk to Human Health

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## ABSTRACT

*Escherichia coli* are known as commensal bacteria in the digestive tract of animals. It is an indicator of faecal contamination when detected in milk, and may be a sign of defective hygiene practices along the processing points. This study aimed to determine the prevalence, molecular characterization, and antimicrobial resistance profiles of *Escherichia coli* isolated from raw cow milk at selected milk collection centers. The overall prevalence of *E.coli* in raw milk was 26.7%. The prevalence in the three areas was; Nyakabirizi (42%), Ishaka TC (30%), and Bushenyi TC (10%) with a p-value of 0.022, which is statistically significant to  $p < 0.05$ , and the posthoc test also showed a significant difference with Nyakabirizi. Of the 23 isolates, 91.3% were susceptible to both chloramphenicol and nalidixic acids. Resistance was observed with trimethoprim-sulfamethoxazole (47%), ampicillin (39.1%), and tetracycline (30.4%). Molecular characterization indicated one sample was positive for the H7 flagella gene after PCR and sequencing.

**Keywords:** Raw milk, *Escherichia coli*, Potential risk, Cow, Human

## INTRODUCTION

Milk is a highly nutritious food recommended to several vulnerable groups, such as young children and expectant mothers [1]. In Sub-Saharan Africa, raw milk is consumed directly by a large number of people in rural areas and indirectly by a much larger segment of the population through the consumption of numerous types of milk products [2]. However, it is prone to contamination by both infectious and non-infectious agents, posing risks to human health. Among some of the infectious agents are *Campylobacter* spps, *Listeria monocytogenes*, *Salmonella* spps, and *E.coli* [3].

*Escherichia coli* (*E. coli*) was first identified as a human pathogen in 1975 from a California patient with diarrhea and was linked to food-borne disease in 1982 [4]. Even though *E.coli* is a known commensal bacterium as part of the intestinal micro-flora of a

variety of animals, not all strains are harmless [5]. Since milk is highly nutritious, several bacteria can multiply within it. The presence of food-borne organisms in milk increases the risk of transmission of food-borne pathogens [6].

Microbes gain entry into milk directly from dairy cows, the farm environment (especially water), equipment used for storage of the milk, and during transportation [7]. Coliforms, *E.coli* inclusive, are commonly referred to as marker organisms [8].

Among the various *E.coli* strains, pathogenic *E.coli* belongs to the first six groups: 1. *Enteropathogenic E.coli* (EPEC) associated with infantile diarrhoea, 2. *Enteroinvasive E.coli* (EIEC) causing dysentery-like disease, 3. *Enterotoxigenic E.coli* (ETEC) produce enterotoxins leading to cases of diarrhea, 4. *Enteroaggregative E.coli* (EAEC) express aggregative adherence, 5. Diffusely adherent *E.coli* (DAEC) that adheres to the surface of epithelial

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**Received:** 29-Jul-2024, Manuscript No. JBP-24-26611; **Editor assigned:** 31-Jul-2024, Pre QC No. JBP-24-26611 (PQ); **Reviewed:** 14-Aug-2024, QC No. JBP-24-26611; **Revised:** 21-Aug-2024, Manuscript No. JBP-24-26611 (R); **Published:** 28-Aug-2024, DOI:10.35248/2155-9597.24. S27.101

**Citation:** Pius T, Yashim BJS, Ntulume I, Ssajjakambwe P (2024). Prevalence and Molecular detection of *Escherichia coli* isolated from raw cow milk at selected collection centres in Bushenyi district, Western Uganda: A potential risk to human health. *J Bacteriol Parasitol*. S27:101.

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cells, and 6. Shiga-producing *E.coli* (STEC) which may also be referred to as Verocytotoxin-producing *E.coli* (VTEC) or enterohemorrhagic *E.coli* (EHEC) produces stx1 and stx 2 causing Hemorrhagic Colitis (HC) and sometimes Hemolytic Uremic Syndrome (HUS) in humans [9].

Once the hygiene at production, handling, transportation, and points of sale of milk is compromised, the milk sold in raw form poses a great risk to public health [10]. This study focused on investigating the presence of *E.coli* in milk samples collected at selected milk collection centers in the Bushenyi district. There is a paucity of information about the *E.coli* isolates from milk, with emphasis on the characterization of possible virulence factors at the molecular level.

## MATERIALS AND METHODS

### Study area and design

A cross-sectional study was conducted in the Bushenyi district, where raw milk (10 ml) was collected from each center, making a total of 86 collection centers, stratified into three geographical areas (Ishaka town, Nyakabirizi, and Bushenyi trading center), South-western Uganda. This entailed purposive sampling at those centers that consented to participate in this study.

### Ethical consideration

Informed consent was obtained after a clear explanation of the study objectives and the benefits. The consent form was administered in either English or Runyankole, the most common indigenous language of the area.

### Sample collection and handling

Milk was aseptically collected and placed in sterile sample collection tubes, tightened with a screw cap, and placed on ice in a cool box. The collected samples were transported to the Kampala International University microbiology laboratory for further analysis. Each sample was assigned a serial number immediately after collection, which also concealed the identity of the collection centres to respect the confidentiality agreement in the consent form.

### Microbiology culture and isolation

Standard microbiology procedures were employed to run the individual samples. Inoculated plates using the streaking method on VRBA, and MacConkey agar were incubated at 37°C for a maximum period of 48 hours. Bacterial colonies were identified by their common phenotypic characteristics culturally. In addition, conventional biochemical methods were applied to the isolates for further identification of the *E.coli* isolates.

Briefly, all sample manipulations and preparations were done under a Class 2 biosafety cabinet (Esco, Singapore). A modification of the method used, samples were first enriched in buffered peptone water broth (Oxoid, UK) at 37°C for 24 hours [11]. The overnight bacterial broths were then streaked onto MacConkey Agar (Conda, Spain) and incubated at 37°C for 24 hours. Presumptive *E.coli* colonies on MacConkey Agar were

medium-sized, intense pink (red) in color, and surrounded by a zone of precipitated bile acids. A single colony was further gram-stained and sub-cultured on Nutrient Agar to obtain a pure culture before biochemical testing. Biochemical analysis of the *E.coli* isolates was done using indole, methyl red, lactose and citrate utilization tests.

### Sensitivity analysis

Employing the disc diffusion method with modifications, the inocula of isolated strains was prepared in 1% peptone water. Adjustments of the turbidity equal to 0.5 McFarland standard were executed, thereafter, a sample was applied onto Mueller Hinton agar using a sterile swab to spread the culture solution onto the surface of solid media. The inoculated plate was left to dry in a biosafety cabinet for 5 minutes. Subsequently, antibiotic sensitivity discs were applied onto the inoculated agar using sterile forceps and placed in a 37°C incubator. Zones of inhibition were measured in mm with the help of a ruler after 18-24 hours of incubation and findings were compared against a reference chart. The antibiotics used were ampicillin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), neomycin (30 µg), nalidixic acid (30 µg) and trimethoprim-sulfamethoxazole (5 µg) [12].

### Polymerisation chain reaction

A conventional Polymerase Chain Reaction (PCR) test was run for the *E.coli* isolates to check for the presence of the gene specifically coding for the flagella antigen H7. The primer sequences were Forward-GCGCTGTCGAGTTCTATCGAGC and Reverse-AACGGTGACTTTATCGCCATTCC, adopted and the expected band size was 625 base pairs [13]. For a 25 µl reaction volume, 15 µl of a one Taq PCR master mix was used as per manufacturer's instructions, with 1 µl of 10 µM of each primer, 1 µl DNA template and 7 µl of PCR grade water [2,15]. The initial denaturation temperature was 95°C for 5 minutes, followed by denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and 72°C for 1 minute, all run for 30 cycles. The final extension was at 72°C for 10 minutes and a holding temperature of 4°C. The PCR products were loaded on an agarose gel and viewed under UV light after electrophoresis [14].

## RESULTS

### Prevalence

In this study, selected collection centers indicated the presence of *E.coli* in the milk (a threat to human health), with a p-value of 0.02, which is a statistically significant finding (Table 1).

### Colony-Forming Units (CFUs)

The Colony Forming Unit (cfu) enumeration revealed the total bacteria counts of the different study areas with Bushenyi and Nyakabiizi districts having the highest counts above the internationally acceptable standard of  $3.62 \times 10^7 \pm 1.37 \times 10^7$  cfu/ml (Table 2).

### Antimicrobial resistance profiles

Among the isolates, ampicillin and tetracycline showed the highest resistance as seen (Table 3 and Figure 1).

Study areas	No. of Samples	Prevalence (%)	95% CI		P value
			LL	UP	
Ishaka TC	30	9 (30)	14.73	49.40	0.02
Nyakabirizi	26	11 (42.3)	23.37	63.08	
Bushenyi TC	30	3 (10)	2.11	26.53	
Total	86	23 (26.74)	17.77	37.38	

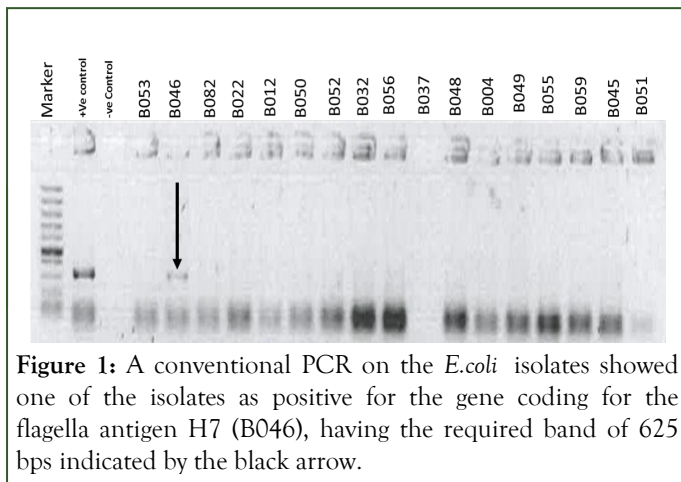
**Table 1:** Presence of *E.coli* in the milk in selected collection centers.

Sampling areas	Total aerobic counts (cfu/ml)				One sample t-test	
	Minimum	Maximum	Mean $\pm$ SD	95% CI	t-value	P-value
	CFUs (x10 <sup>7</sup> )					
Ishaka TC	0.026	19.3	2.39 $\pm$ 3.61a	1.037-3.735	3.617	0.001
Nyakabirizi	0.029	17.3	6.15 $\pm$ 5.48b	3.929-8.357	5.716	<0.0001
Bushenyi TC	0.011	58.0	6.17 $\pm$ 1.29b	1.362-10.981	2.624	0.014

**Table 2:** Multiple Tukey comparison test with significant differences ( $p < 0.05$ ) represented by different superscripts (a, b). TC- Trading center.

Antibiotic used	Number of <i>E.coli</i> isolates (n=23)		
	Susceptible	Intermediate	Resistant
Ampicillin (30 $\mu$ g)	7 (30.4%)	7 (30.4%)	9 (39.1%)
Tetracycline (30 $\mu$ g)	9 (39.1%)	7 (30.4%)	7 (30.4%)
Neomycin (30 $\mu$ g)	0 (0%)	18 (78.3%)	5 (21.7%)
Chloramphenicol (30 $\mu$ g)	21 (91.3%)	0 (0%)	2 (8.7%)
Nalidixic acid (30 $\mu$ g)	21 (91.3%)	2 (8.7%)	0 (0%)
Trimethoprim-sulfamethoxazole (5 $\mu$ g)	12 (52.2%)	0 (0%)	11 (47.8%)

**Table 3:** Antibiotic used for *E.coli* isolates.



**Figure 1:** A conventional PCR on the *E.coli* isolates showed one of the isolates as positive for the gene coding for the flagella antigen H7 (B046), having the required band of 625 bps indicated by the black arrow.

## DISCUSSION

Milk is an essential supporting medium for microbial growth. Food-borne infections are caused by a wide range of microbial agents, such as *Campylobacter*, *Salmonella*, *Escherichia*, *Brucella*, and *Staphylococcus*, among others which result in mild or life-threatening illnesses. The main objective of this study was to carry out a baseline survey to assess the possible extent of *E.coli* contamination of cow milk at selected collection centers in the Bushenyi district. This was against the backdrop that consumption of unpasteurized milk either directly or indirectly had been reported to be a threat to human health [15].

In this study, *E.coli* was isolated from raw milk, (Table 1). Such a finding is suggestive of fecal contamination, from either water, the environment, or the animals. Another study suggested that contamination of milk and its products had a bearing on the housing conditions, and hygiene standards at milk handling, transportation, and storage [16]. This implied a potential for food-borne illness among consumers of the affected raw milk was eminent. Similar studies revealed prevalence ranging from 7%-90% [17-19].

The CFU counts of Bushenyi and Nyakabirizi districts (Table 2) were above the acceptable cutoff ( $3.62 \times 10^7 \pm 1.37 \times 10^7$  cfu/ml.). For milk, thus a further indication of a threat to human health just in case such milk is not cooked well to kill the bacteria present before consumption. Oltramari et al., and Ribeiro et al., revealed high bacteria counts, this attributable to poor hygiene [2,20]. Furthermore, a pilot study before this work observed that the absence of a milking pailer, and scarcity of clean water among others could also contribute to the above findings. In addition, it was noted that the injudicious use of antibiotics by farmers to treat a range of conditions even without appropriate professional guidance from a veterinarian was a common practice. The latter was raised thus a fertile ground to contribute to the development of antibiotic resistance [21]. The trends in (Table 3) were no surprise, with the major resistance noted against ampicillin and tetracycline. Tetracycline is one of the most abused drugs by livestock communities, whereby it is used to treat a range of infections. Since the responses are quite commendable, it is a common drill to carry this drug in the first aid kit of many farmers. The use of tetracycline for prophylactic purposes by these communities is the order of the day. A study

showed a resistance of 100% of *E.coli* to tetracycline [22]. These two drugs are very common antibiotics that farmers use indiscriminately in the forms of penstrep and oxytetracycline to treat almost any thought of infection in animals. That said, the likely development of resistance by the prevalent bacteria may not be uncommon.

Furthermore, molecular characterization to investigate the presence of the gene in the *E.coli* isolates that codes for the flagella antigen H7 was performed. Only one sample (B046) was positive. Also to note, earlier and similar work by Msolo et al., and Lye et al., identified this gene that codes for the organelle, the flagella used for motility and adherence onto host cells [5,19]. It's known that *E.coli* strains that possess an active gene coding for the flagella antigen H7 are more virulent than those that do not express this gene. This further implies that once humans consume milk from the B046 collection center, chances of developing food-borne infections are high once the individuals' milk is not well prepared, and also if all the host immune system is compromised, such as in HIV/AIDS patients.

## CONCLUSION

The presence of *E.coli* in milk, especially in amounts above the accepted, and also if the isolates do possess known virulence factors as a flagellum, poses a risk to human health. This calls for appropriate preparation of food before consumption in addition to adherence to the required hygiene standards of handling and processing of food destined for human consumption. Additionally, the use of antibiotics after the guidance of a trained medical practitioner is highly recommended.

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